Amendments to the Specification

Please replace the paragraph from page 3, lines 10-21 with the following replacement paragraph:

During the early moments of reperfusion and/or inflammation, in response to oxygen radical species, the serine protease thrombin, histamine, tumor necrosis factor-alpha (~~TNF~~TNFα), platelet activating factor, and IL-1, the pro-adhesive properties of endothelium are stimulated [16-19]. P-selectin, stored as preformed granules in the Weibel-Palade bodies, is rapidly translocated to the endothelial surface [21-23]. Interaction with P-selectin on endothelium causes the neutrophil to start rolling and attaching loosely on the endothelial surface [17, 24]. This "rolling phenomenon" plays a critical role in the pathogenesis of the early phase of reperfusion injury in myocardium [25]. These same factors are also known stimulants of tissue factor. The endothelium may be further stimulated by thrombin generated by tissue factor localized on its cell surface, by neutrophils/monocytes circulating in the region, and by myocytes [20].

Please replace the paragraph from page 4, lines 1-8 with the following replacement paragraph:

After the initial tethering of PMNs to the vascular endothelium, firm adherence is facilitated by interaction between CD11b/CD18 on PMNs and ICAM-1 on the endothelium. ICAM-1 is constitutively expressed at low levels, but *de novo* protein synthesis and surface expression is stimulated by cytokines (e.g., ~~TNF~~TNFα) beginning at 4-6 hours after reperfusion, and peaking at 24 hours. Studies confirm that endothelial ICAM-1 is not significantly expressed until between 6 and 24 hours of reperfusion, with expression in myocytes occurring later than 24-72 hours [15, 34]. This later response is in contrast to the early (<30 minutes) expression of P-selectin.

Please replace the paragraph from page 9, lines 7-20 with the following replacement paragraph:

Based on adenosine's potent inhibition of neutrophil-mediated reperfusion injury, Thourani et al. [53] tested the hypothesis that adenosine given during the period of reperfusion following aortic declamping would provide similar benefits to adenosine administered as an adjunct to blood cardioplegia. In a canine model of regional coronary occlusion, it was shown that adenosine administered either as an adjunct to blood cardioplegia (~~100 □M~~100 μM) alone or only during reperfusion (140 μg/kg/min) reduced infarct size, which is further described *infra* in connection with FIG. 4, improved post-ischemic contractile function, reduced myocardial edema, and attenuated neutrophil accumulation in the ischemia-reperfused area compared to the unsupplemented blood cardioplegia group. Furthermore, the hearts treated with adenosine only during reperfusion demonstrated better post-ischemic coronary artery endothelial function that was not observed with either unsupplemented blood cardioplegia or adenosine-enhanced blood cardioplegia. This observation is consistent with adenosine's potent anti-neutrophil effects.

Please replace the paragraph from page 9, lines 29-31, through page 10, lines 1-11 with the following replacement paragraph:

Unlike adenosine, aprotinin is a potent inhibitor of serine protease activity, including kallikrein, and thrombin. In a porcine closed-chest model of LAD occlusion and reperfusion, thrombin levels increased specifically during the reperfusion phase. In addition to its effects on the coagulation cascade, thrombin is a direct activator of P-selectin expression on coronary vascular endothelial cells, which initiates the recruitment of neutrophils and other inflammatory cells in the pathogenesis of reperfusion injury [3]. Thrombin also stimulates platelets, which release cytokines that activate neutrophils, in addition to directly binding to neutrophils, thereby further supporting thrombin as a potential participant in the inflammatory response involving neutrophils. Studies support the hypothesis that thrombin may be a mediator of reperfusion injury through activation of coronary vascular endothelium, or by stimulating the generation of cytokines such as ~~TNF□~~TNFα [4]. Although aprotinin inhibits the extravasation of neutrophils, it does not inhibit early neutrophil adherence to coronary artery endothelium [12].

Please replace the paragraph from page 11, lines 20-31, through page 12, lines 1-8 with the following replacement paragraph:

A few studies have reported that aprotinin used in conjunction with a cardioplegic solution setting, particularly for long-term storage. In a study by Sunamori et al. [61], isolated canine hearts were administered multidose (q 1hour) crystalloid cardioplegia containing 150 KIU aprotinin for 6 hours of arrest, followed by 2 hours of blood reperfusion from a donor system. There was no difference between the aprotinin-treated hearts and a control group in post-ischemic non-specific creatine kinase (CK) activity or CK-MB isoenzyme activity. Recovery of post-ischemic ATP after reperfusion was significantly greater in the aprotinin-treated group, with no differences in other high-energy phosphates. However, levels of the lysosomal enzyme ~~*N*-acetyl-□-*D*-glucosaminidase~~*N*-acetyl-β-*D*-glucosaminidase in coronary sinus blood were significantly lower during reperfusion in the aprotinin group. In addition, morphologic damage was moderate in the control group, while it was largely minimal in the aprotinin group. Paradoxically, there was significantly less recovery of systolic function (end-systolic pressure-volume relationship) in the aprotinin-treated group. In summary, no clear picture of myocardial preservation was demonstrated in this study [61]. In contrast to the study of Sunamori et al. [61], Gurevitch et al. [62] showed significant protection with 105 KIU/L aprotinin administered as a pretreatment and adjunct to crystalloid cardioplegia. These conflicting data suggest that dose of aprotinin used is an important factor, and suggests the need for carefully conducted dose-response studies.

Please replace the paragraph from page 12, lines 9-23 with the following replacement paragraph:

In a model of storage in cold cardioplegia, Bull et al. [63] reported that 200 KIU aprotinin attenuated the decline in ATP content and protein synthesis of rat myocardium slices incubated in cold (4°C) crystalloid cardioplegia solution for up to 6 hours of storage. It was not clear from that study whether aprotinin maintained greater ATP concentration by improving myocardial synthesis of ATP, or by the reduction in the hydrolysis of ATP. Importantly, this study [63]

reported that aprotinin suppressed both ~~TNF□~~TNFα generation and uptake by the myocardial tissue slices. These beneficial effects were achieved in a system free of inflammatory cells and plasma soluble elements, such as circulating thrombin, FVII/a, etc. The study of Gurevitch et al. [62] confirmed cardioprotection by high-dose aprotinin in a blood cell-free and plasma-free model of ischemia and reperfusion. There were several magnitudes of difference in the concentration of aprotinin used between the two studies. Again, the effective dose of aprotinin necessary to demonstrate cardioprotection during cardioplegia, either acutely or during prolonged cold storage, remains to be identified.

Please replace the paragraph from page 13, lines 7-18 with the following replacement paragraph:

The serine protease inhibitor is selected from the group consisting of 4-(2-aminoethyl) benzenesulfonylfluoride, ~~□-amino-*n*-caproic acid~~ε-amino-*n*-caproic acid, ~~□$_1$-antichymotrypsin~~α_1-antichymotrypsin, antipain, antithrombin III, ~~□$_1$-antitrypsin~~α_1-antitrypsin, *p*-amidinophenylmethylsulfonyl fluoride, aprotinin, cathepsin/subtilisin inhibitor (Boc-Val-Phe-NHO-Bz-*p*Cl), chymostatin ~~([(*S*)-1-carboxy-2-phenylethyl]-carbamoyl-□-[2-amidohexahydro-4(*S*)-pyrimidyl]-(*S*)-glycyl-[A = Leu, B = Val, or C = Ile]-phenylalaninal)~~([(*S*)-1-carboxy-2-phenylethyl]-carbamoyl-α-[2-amidohexahydro-4(*S*)-pyrimidyl]-(*S*)-glycyl-[A = Leu, B = Val, or C = Ile]-phenylalaninal), chymotrypsin inhibitor I, 3,4-dichloroisocoumarin, diisopropylfluorophosphate, dipeptidylpeptidase IV inhibitor I (Ile-Pro-Ile), dipeptidylpeptidase IV inhibitor II (H-Glu-(NHO-Bz)-Pyr), ecotin, elastase inhibitor I (Boc-Ala-Ala-Ala-NHO-Bz), ~~□$_2$-macroglobulin~~α_2-macroglobulin, PPACK (*D*-Phe-Pro-Arg-chloromethylketone), PPACK II, N^a-tosyl-Lys chloromethyl ketone, N^a-tosyl-Phe chloromethyl ketone, and any mixture thereof. In a preferred embodiment, the serine protease inhibitor is aprotinin.

Please replace the paragraph from page 15, lines 18-31, through page 17, lines 1-10 with the following replacement paragraph:

In one embodiment of the present invention, the protease inhibitor is selected from the

group consisting of 4-(2-aminoethyl) benzenesulfonylfluoride, ~~□-amino-*n*-caproic acid~~ε-amino-*n*-caproic acid, ~~□$_1$-antichymotrypsin~~α_1-antichymotrypsin, antipain, antithrombin III, ~~□$_1$-antitrypsin~~α_1-antitrypsin, *p*-amidinophenylmethyl sulfonyl fluoride, aprotinin, cathepsin/subtilisin inhibitor (Boc-Val-Phe-NHO-Bz-*p*Cl), chymostatin ~~([(*S*)-1-carboxy-2-phenylethyl]-carbamoyl-□-[2-amidohexahydro-4(*S*)-pyrimidyl]-(*S*)-glycyl-[A = Leu, B = Val, or C = Ile]-phenylalaninal)~~([(*S*)-1-carboxy-2-phenylethyl]-carbamoyl-α-[2-amidohexahydro-4(*S*)-pyrimidyl]-(*S*)-glycyl-[A = Leu, B = Val, or C = Ile]-phenylalaninal), chymotrypsin inhibitor I, 3,4-dichloroisocoumarin, diisopropylfluoro phosphate, dipeptidylpeptidase IV inhibitor I (Ile-Pro-Ile), dipeptidylpeptidase IV inhibitor II (H-Glu-(NHO-Bz)-Pyr), ecotin, elastase inhibitor I (Boc-Ala-Ala-Ala-NHO-Bz), ~~□$_2$-macroglobulin~~α_2-macroglobulin, PPACK (*D*-Phe-Pro-Arg-chloromethylketone), PPACK II, N^a-tosyl-Lys chloromethyl ketone, N^a-tosyl-Phe chloromethyl ketone, acetyl-pepstatin (Ac-Val-Val-(3*S*,4*S*)-Sta-Ala-(3*S*,4*S*)-Sta-OH), calpain inhibitor I (*N*-acetyl-Leu-Leu-norleucinal), calpain inhibitor II (*N*-acetyl-Leu-Leu-Met-CHO), amastatin ([(2*S*, 2*R*)]-3-amino-2-hydroxy-5-methylhexanoyl]-Val-Val-Asp-OH), arphamenine A ((2*R*,5*S*)-5-amino-8-guanidino-4-oxo-2-phenylmethyloctanoic acid), arphamenine B ((2*R*,5*S*)-5-amino-8-guanidino-4-oxo-2-*p*-hydroxyphenylmethyloctanoic acid), benzamidine, bestatin ([(2*S*, 2*R*)-3-amino-2-hydroxy-4-phenylbutanoyl]-*L*-Leucine), CA-074 ((*L*-3-*trans*-[propylcarbamoyl]oxirane-2-carbonyl)-*L*-isoleucyl-*L*-proline), CA-074-Me ((*L*-3-*trans*-[propylcarbamoyl]oxirane-2-carbonyl)-*L*-isoleucyl-*L*-proline-methylester), calpastatin, calpeptin (benzyloxycarbonylleucyl-norleucinal), carboxypeptidase inhibitor, cathepsin inhibitor I (*Z*-Phe-Gly-NHO-Bz), cathepsin inhibitor II (*Z*-Phe-Gly-NHO-Bz-*p*Me), cathepsin inhibitor III (*Z*-Phe-Gly-NHO-Bz-*p*OMe), cathepsin B inhibitor I (*Z*-Phe-Ala-CH_2F), cathepsin B inhibitor II (Ac-Leu-Val-lysinal), cathepsin L inhibitor I (*Z*-Phe-Phe- CH_2F), cathepsin L inhibitor II (*Z*-Phe-Tyr-CHO), cathepsin L inhibitor III (*Z*-Phe-Tyr-(*t*-Bu)-CHN_2), cathepsin L inhibitor IV (1-naphthalenesulfonyl-Ile-Trp-CHO), cathepsin L inhibitor V (*Z*-Phe-Tyr(O*t*Bu)-COCHO), cathepsin L inhibitor VI ~~(*N*-(4-biphenylacetyl)-*S*-methylcysteine-(*D*)-Arg-Phe-□-phenethylamide)~~(*N*-(4-biphenylacetyl)-*S*-methylcysteine-(*D*)-Arg-Phe-β-phenethylamide), cathepsin S inhibitor (*Z*-Phe-Leu-COCHO), cystatin, diprotin A (H-Ile-Pro-Ile-OH), E-64 (*trans*-epoxysuccinyl-*L*-leucylamido-(4-guanidino)butane), E-64 d (loxistatin, or (2*S*,3*S*)-*trans*-epoxysuccinyl-*L*-leucylamido-3-methylbutane ethyl ester), ebelactone A (3,11-dihydroxy-

2,4,6,8,10,12-hexamethyl-9-oxo-6-tetradecenoic 1,3-lactone), ebelactone B (2-ethyl-3,11-dihydroxy-4,6,8,10,12-penta methyl -9-oxo-6-tetradecenoic 1,3-lactone), EDTA (ethylenediaminetetraacetic acid), EGTA ~~(ethyleneglycol-*bis*(□-aminoethyl)-*N,N,N',N'*-tetraacetic acid)~~(ethyleneglycol-*bis*(β-aminoethyl)-*N,N,N',N'*-tetraacetic acid), elastase inhibitor II (MeOSuc-Ala-Ala-Pro-Ala-CMK), elastase inhibitor III (MeOSuc-Ala-Ala-Pro-Val-CMK), elastatinal (Leu-(Cap)-Gln-Ala-al or *N*-[(*S*)-1-carboxy-isopentyl)-carbamoyl-alpha-(2-iminohexahydro-4(*S*)-pyrimidyl]-*L*-glycyl-*L*-glutaminyl-*L*-alaninal), E-64 (loxistatin, or (2*S*,3*S*)-*trans*-epoxysuccinyl-*L*-leucylamido-3-methylbutane ethyl ester), *N*-ethyl maleimide, GGACK (1,5-dansyl-*L*-glutamyl-*L*-glycyl-*L*-arginine chloromethyl ketone), galardin (*N*-[(2*S*)-(methoxycarbonylmethyl)-4-methylpentanoyl]-*L*-tryptophan-methyl amide), 2-guanidinoethylmercaptosuccinic acid, hirudin, HIV protease inhibitor (Ac-Leu-Val-phenylalaninal), leuhistin (((2*R*,3*S*)-3-amino-2-hydroxy-2-(1H-imidazol-4-ylmethyl)-5-methyl)-5-methylhexanoic acid), leupeptin (acetyl-leucyl-leucyl-arginal), NCO-700, PEFABLOC SC (4-(2-aminoethyl)-benzenesulfonyl fluoride), pepstatin (isovaleryl-Val-Val-4-amino-3-hydroxy-6-methylheptanoyl-Ala-4-amino-3-hydroxy-6-methylheptanoic acid), phebestin ((2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutanoyl-*L*-valyl-*L*-phenylalanine), PMSF (phenyl methyl sulfonyl fluoride), phosphoramidon (*N*-alpha-*L*-rhamnopyranosyloxy(hydroxyl phosphinyl)-*L*-Leucyl-*L*-tryptophan, plummer's inhibitor (*D,L*-2-mercaptomethyl-3-guanidino-ethylthiopropanoic acid), 1,10-phenanthroline, subtilisin inhibitor I (Boc-Ala-Ala-NHO-Bz), subtilisin inhibitor II (*Z*-Gly-Phe-NHO-Bz), subtilisin inhibitor III (*Z*-Gly-Phe-NHO-Bz-*p*OMe), subtilisin inhibitor IV (Boc-Pro-Phe-NHO-Bz-*p*Cl), subtilisin inhibitor V (Boc-Ala-Pro-Phe-NHO-Bz), TIMP-2 (tissue inhibitor of metalloproteinase 2), trypsin inhibitor, secretory leukocyte protease inhibitor, and any mixture thereof.